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IN-VITRO SHOOT TIP CULTURE OF ASHWAGANDHA (*Withania somnifera*)

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Abstract: Micropropagation of Ashwagandha was done via the process of Plant Shoot tip Tissue Culture techniques where the explants were taken as shoot tips from stock plants of ashwagandha, and growth hormone was taken as BAP, in nutrient media of Murashige and Skoog. Five nutrient medias were prepared using five different hormone concentrations of BAP (2mg/L, 2.5mg/L, 3mg/L, 3.5mg/L, 4mg/L). Ten shoot tips were taken from stock plants and surface sterilised for inoculation in the media jars (2 explants per jar). After inoculation, the inoculated explants were incubated in the incubation room in photoperiod racks. The room temperature was maintained at 24-28 degrees celsius, where 17 hours of daytime and 7 hours of night time were provided to them.The explants were kept in incubation for 25 days, and it was observed that the explants which were inoculated in the media with hormone concentration of 3mg/L, responded the best, providing a total of 10 branches and final length of 4.7 centimetres, from the initial stage of having 3 branches and 2.1 centimetres in length.

Keywords- **ASHWAGANDHA, BAP, MICROPROPAGATION, SHOOT TIP, *WITHANIA SOMNIFERA***

I.INTRODUCTION

Ashwagandha (*Withania somnifera*)is an Ayurvedic herb, belonging to the family of solanaceae. This plant is also known as Indian Ginseng or Indian Winter Cherry, which is native to the Indian Subcontinent Nepal and Pakistan, specially in the dry subtropical regions like Rajasthan, Punjab, Haryana, Madhya Pradesh, Gujarat, Maharashtra [1]. Ashwagandha is therapeutically important, as it has shown adaptogenic properties to promote well-being by adapting to stress[2]. It is medically important as it contains biologically active compounds like flavonoids, alkaloids, lactones (withanolids) and saponins[3]. These compounds have shown anti-inflammatory, antioxidant, immunomodulatory, neuroprotective and anti stress effects In humans. It has been also reported that ashwagandha has some anti-cancer, anti-anxiety, anti-diabetic and anti-depressive properties as well[4]. Since this plant has such a vast range of therapeutic properties and medicinal properties,

further research on this plant is crucial, and there comes the utility of tissue culture. For the process of tissue culture of ashwagandha, at first the explant needs to be collected, like shoot tip, leaf segments or axillary buds (For this paper, shoot tip was chosen as the appropriate explant). Then these explants are surface sterilised via using surface sterilants like tween 20, bavistine and mercuric chloride, to remove any form of microbial contaminants[5][6]. Then these sterilised explants are cultured in a nutrient media containing the specific amount of growth hormones like auxin and cytokinin. Then those explants are incubated under controlled environmental conditions where they go through cell division, organogenesis and finally turn into a complete plant. Tissue culture of Ashwagandha has its privileges of of allowing rapid multiplication of plants, producing a disease free stock, and preserving the valuable genotype, all of which are not compilable with conventional methods. Moreover, tissue culture can be used to enhance production of secondary metabolites like withanolids by manipulation the growth regulators and by precursor feeding, the opportunity of research with Ashwagandha via tissue culture is vast[7]. Through various literature, it has been observed that many scientists have done wonderful research in different kind of ways to achieve various scientific milestones based on Ashwagandha. In one study, the researchers produce a comprehensive review on the current progress which has been achieved on the various biotechnological and tissue culture aspects of ashwagandha and to cover the latest information related to in vitro tissue culture for the same for the production of withanolids[8]. Another study talks about how micropropagation of ashwagandha is so crucial to meet the demand of that plant for commercial use and for that it sheds light on how important is to optimise the number of factors such as nutrient medium, status of medium (solid and liquid), type of explant, and plant growth regulators, for the rapid growth of the explant[9]. Another research article concentrates on micro propagating the nodal explants through in vitro-procedures and then the articles goes into detail about how the regenerated micro shoots roots best when put in 1/2 MS medium containing NAA, when established in earthen pots containing garden soil, gave 95% survival rate[10]. In another experiment, researchers used undefined media containing 10% coconut milk to witness an increase in biomass of the shoot tip explant by 37 fold[11]. A variety of research has been done on Ashwagandha based on extraction of metabolites and on finding better ways for propagation of the plant via in-vitro procedures using nodes, shoot tips as explants, and via procedures of callus formation, but still further research is necessary on the topic of micropropagation of Ashwagandha, where this research article hopes to contribute its results.

II.MATERIALS AND METHODS

Few of the Ashwagandha plants were collected from the Patuli Flower market, Kolkata, as stock plants and were kept under observation to let the saplings grow, and produce a few more branches, and to screen the plants for any contamination problems (if any). The hardiest plants were chosen for the collection of explants. Glasswares and instruments were sterilised via chromic acid (not used for metal instruments and plastic caps), detergent water, hot air oven and autoclave. The media prepared were five Murashige and Skoog medias in sterilised culture jars with BAP (6-benzylaminopurine) hormone concentrations of 2mg/L, 2.5mg/L, 3mg/L, 3.5mg/L and 4mg/L respectively, and the concentration of Agar used was 8g/L. After sterilisation of the culture bottles along with the media, they were set aside for the agar to solidify. Next the explants (shoot tips) were excised from the selected stock plants and surface sterilised with 2-3 drops of tween 20 per 100ml

distilled water, 0.15gm of Bavisitne per 100ml distilled water and a 1ml solution of 0.06g mercuric chloride per 99ml of distilled water. After adding each of the above mentioned surface sterilants, the explants were swirled slowly in them for 6minutes each post which the explants were washed with sterile water three times. After surface sterilisation of the explants, the lower exposed part of each explant was removed via a sterile scalpel and then two explants each were inoculated in the previously prepared culture jars (Entire process of surface sterilisation and inoculation was done inside a sterilised laminar air flow cabinet). After inoculation, the jars were transferred into the incubation room, in the photoperiod racks. The artificial environment was set to provide 17 hours of daytime and 7 hours of night time with the temperature maintained at 24-28 degrees celsius. The explants were kept in incubation for 25days and observed for growth and development.

III.RESULT

Five culture jars containing two explants each for incubation were prepared and inoculated. The five jars being:

Explant A with media containing BAP in a concentration of 2mg/litre

Explant B with media containing BAP in a concentration of 2.5mg/litre

Explant C with media containing BAP in a concentration of 3mg/litre

Explant D with media containing BAP in a concentration of 3.5mg/litre

Explant E with media containing BAP in a concentration of 4mg/litre

Each of the five culture jars were observed in the morning under the light of the photoperiod racks and then the measurements of each of the explants were taken and recorded. This process was repeated for 25 days.

EXPLANT A (BAP 2mg/litre)

NUMBER OF DAYS	LENGHT OF SHOOT IN (cm)	NUMBER OF BRANCHES
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2
8	2.2	2
9	2.2	2
10	2.2	2
11	2.3	2
12	2.3	3
13	2.3	3
14	2.3	3
15	2.3	3
16	2.4	3
17	2.4	3
18	2.5	3
19	2.5	3
20	2.5	3
21	2.5	4
22	2.5	4
23	2.6	4
24	2.6	4
25	2.6	4

Fig 1: Obs. of Explant

EXPLANT B (BAP 2.5mg/litre)

NUMBER OF DAYS	LENGHT OF SHOOT IN (cm)	NUMBER OF BRANCHES
1	2.3	3.0
2	2.3	3.0
3	2.3	3.0
4	2.4	3.0
5	2.4	3.0
6	2.4	3.0
7	2.5	3.0
8	2.5	3.0
9	2.5	3.0
10	2.6	4.0
11	2.6	4.0
12	2.6	4.0
13	2.7	4.0
14	2.7	4.0
15	2.7	4.0
16	2.7	4.0
17	2.9	5.0
18	2.9	5.0
19	3.1	5.0
20	3.1	5.0
21	3.2	5.0
22	3.4	6.0
23	3.4	6.0
24	3.4	6.0
25	3.6	6.0

Fig 2: Obs. of Explant B

EXPLANT C (BAP 3mg/litre)

NUMBER OF DAYS	LENGHT OF SHOOT IN (cm)	NUMBER OF BRANCHES
1	2.1	3.0
2	2.1	3.0
3	2.1	3.0
4	2.3	3.0
5	2.3	3.0
6	2.4	3.0
7	2.5	3.0
8	2.7	4.0
9	2.7	4.0
10	2.8	4.0
11	2.9	6.0
12	3.2	7.0
13	3.5	7.0
14	3.8	7.0
15	4.0	9.0
16	4.0	10.0
17	4.2	10.0
18	4.5	10.0
19	4.5	10.0
20	4.5	10.0
21	4.6	10.0
22	4.6	10.0
23	4.6	10.0
24	4.7	10.0
25	4.7	10.0

Fig 3: Obs. of Explant C

EXPLANT D (BAP 3.5mg/litre)

NUMBER OF DAYS	LENGHT OF SHOOT IN (cm)	NUMBER OF BRANCHES
1	2.5	2.0
2	2.5	2.0
3	2.5	2.0
4	2.5	2.0
5	2.6	2.0
6	2.6	2.0
7	2.6	2.0
8	2.6	3.0
9	2.7	3.0
10	2.7	3.0
11	2.8	3.0
12	2.8	3.0
13	2.9	4.0
14	3.0	4.0
15	3.0	4.0
16	3.1	4.0
17	3.1	4.0
18	3.1	4.0
19	3.1	4.0
20	3.1	5.0
21	3.3	5.0
22	3.4	5.0
23	3.4	5.0
24	3.4	5.0
25	3.4	6.0

Fig 4: Obs. of Explant D

EXPLANT E (BAP 4mg/litre)

NUMBER OF DAYS	LENGHT OF SHOOT IN (cm)	NUMBER OF BRANCHES
1	2.2	1.0
2	2.2	1.0
3	2.2	1.0
4	2.3	1.0
5	2.4	1.0
6	2.4	1.0
7	2.4	2.0
8	2.5	2.0
9	2.5	2.0
10	2.7	2.0
11	2.7	2.0
12	2.7	3.0
13	2.9	3.0
14	2.9	3.0
15	3.1	3.0
16	3.1	3.0
17	3.1	3.0
18	3.2	4.0
19	3.2	4.0
20	3.2	4.0
21	3.2	4.0
22	3.3	4.0
23	3.3	5.0
24	3.3	5.0
25	3.3	5.0

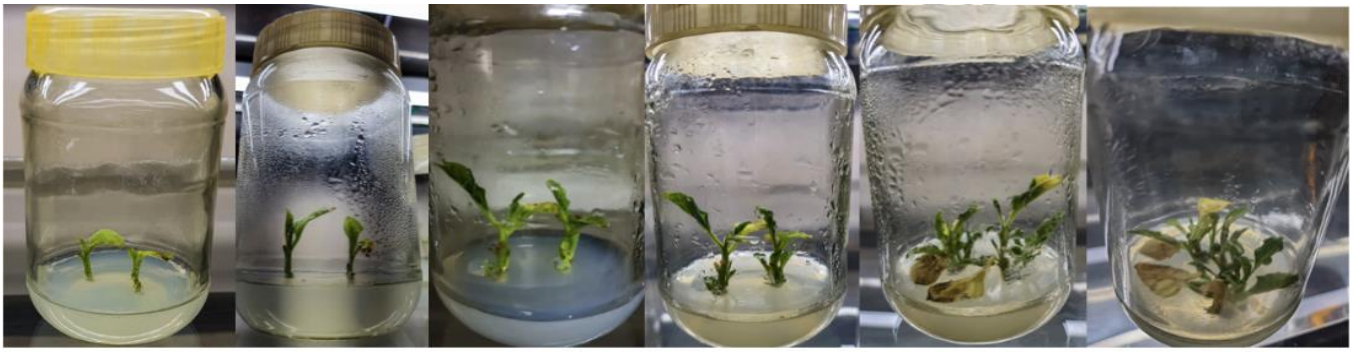
Fig 5: Obs. of Explant E

IV. DISCUSSION

After evaluation of the data acquired from the above datasets, it is seen that in the fixed time period of 25 days, Explant C, I.e., the explants inoculated in the jar containing BAP with a concentration of 3mg/litre has shown the highest growth in length (4.7cm) as well as has shown the production of the maximum number of branches (10 branches), from initially having 3 branches and an initial length of 2.1centimetres.

Note: The data is based on the explant of each jar which has shown the maximum growth.

This experiment was performed using a solid media using agar as a solidification agent, there have been other experiments which have observed that the state of the media and the choice of explant also affect the overall growth of the explant[9]. There also have been other experiments where the scientists have chosen to take undefined media instead of defined media like addition of coconut milk to MS media, to which they have observed a drastic improvement in the growth rate and change in biomass of the explants[11]. Moreover, there have been other experiments where axillary buds have been used as explants with a combination of growth regulators like [6-benzyladenine (BA), kinetin (Kin), 2-isopentenyladenine (2iP), and thidiazuron (TDZ)], which were either used single or with combination of other regulators like α-napthalene acetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA), while using the base nutrient media as Murashige and Skoog, to which they observed a highest of 90% regeneration frequency when using 2.5 µM 6-benzyladenine (BA) and 0.5 µM NAA and 30 g/l sucrose at pH 5.8, which in the end gave 32 shoots[12].



Day 1 Day 4 Day 11 Day 12 Day 18 Day 25

Fig 6: Growth observation of explant C from Day 1 to Day 25

V. CONCLUSION

Ashwagandha (*Withania somnifera*), is a medicinal plant, which is seen to grow at the highest rate in In-vitro conditions via shoot tip culture, when cultured in Murashige and Skoog media with a BAP (6-benzylaminopurine) hormone concentration of 3mg/litre. Concentrations less than 3 resulted in low rate of growth and if the concentration is more than 3, it also showed a lower rate of growth. Along with the hormone conditions, the plant required 17 hours of Day time and 7 hours or night time, while the temperature was being maintained at 24 degrees Celsius in the night and at 28 degrees Celsius during day time. Ashwagandha in general takes a long time to grow in the In Vivo condition, whereases, via In vitro procedure, the two explants produced about 5-6 shoot tips for further subculture in 25days, when growth with help of defined media, containing a single growth regulator.

Other studies have concluded that with help of using undefined media[11] and by using a combination of growth regulators[12], or by using a different part of the plant as an explant like nodal segments[10] or by doing a callus culture or by using the axillary buds as the explant[12], could provide an even more positive outcome in terms of growth and production of secondary metabolites.

Overall, it could be concluded that in-vitro tissue culture with any type of explant of Ashwagandha, provides better and faster results when compared to in-vivo culture which fails to fulfil the demand of the plant due to low seed viability and poor germination of the plant[13].

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VII. REFERENCES

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